HOW THE MICROBIOLOGY LABORATORY CAN IMPROVE ANTIMICROBIAL THERAPY*

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THE microbiology laboratory is unique among clinical laboratories in several important respects. Specimens are obtained from multiple sites or sources; many sites of origin of specimens have their own indigenous microbial flora; and the most likely pathogenic microorganism may vary by site or source of specimen. Procedures used for microbiological examination of specimens vary by site, and few microbiologic tests can provide sensitive and specific results within an hour or two following the specimen's arrival in the laboratory. The number and variety of microbial species reported by the laboratory increase steadily as the result of the discovery of new species, reclassification of old species, and the use of newer devices that allow clinical laboratories to identify species which until a few years ago could be identified only in large reference laboratories. An effort is made to predict outcome of therapy based on *in vitro* susceptibility tests with an ever-expanding number of antibiotics. Finally, few medical schools provide curricula in infectious diseases, and most microbiology curricula are too preoccupied with molecular biology and genetics to teach much about the medical importance of microorganisms. Thus, it is hardly surprising that microbiology reports are poorly utilized and that empiric antimicrobial therapy often replaces microbiologic diagnosis.[1]

Although the extended spectrum of antibacterial activity provided by newer β-lactam agents, particularly when combined with an aminoglycoside, would superficially appear to support empiric therapy and diminish the importance of microbiologic diagnosis, the high cost of these newer agents and their potential adverse side effects, particularly when combined with aminoglycosides, should encourage more rational use of

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more specific, less costly, and potentially less toxic antibiotics based on the isolation, identification, and susceptibility test results provided by the microbiology laboratory.

What can the Clinician Reasonably Expect from the Microbiology Laboratory?

For more rational use of antibiotics it is important to establish what a clinician can reasonably expect from the laboratory. As summarized by Neu[2] in 1978, the laboratory should provide information required to make a clinical decision, issue guidelines for specimen collection (what, when, and how), identify those microorganisms that are clinically relevant, provide susceptibility test results as rapidly and as meaningfully as possible to the most appropriate antibiotics for the organisms isolated and for the site of infection, institute suitable systems for rapid specimen transport and for delivery of results, and keep the clinical staff abreast of changes in the field and in antimicrobial susceptibility patterns. Obviously, the essential ingredient of each link in this chain of events is communication and dialogue between clinician and microbiologist.

To place this entire problem in perspective, a simple request for "culture and sensitivities" is analogous to a request for "blood chemistries," and is insufficiently specific to allow the microbiologist to provide clinically useful information. Moreover, unlike a fasting blood glucose test, which is reported in quantitative units, has a small coefficient of variation, can be related to a normal range of values, and has high predictive values (positive and negative), microbiologic culture is a highly qualitative test for which coefficients of variation and normal value ranges are poorly defined or nonexistent and which often have poor predictive values. To cite a simple example, approximately 50% of children with positive throat cultures for group A streptococci fail to demonstrate significant antibody increases and are therefore simply carriers of this organism. Consequently, though there are few false-negative throat cultures in children with serologically proven group A streptococcal pharyngitis, there are a significant number of false-positive throat cultures and, as a result, a low positive predictive value for this test.

Throat cultures represent one of many types of selective cultures in which only certain microorganisms are specifically sought. The positive and negative predictive values of results of selective cultures, however, vary according to the prevalence of the disease under consideration. For

example, the positive predictive value of a single isolation of *Mycobacterium tuberculosis* from a patient's sputum is obviously high because, to the best of anyone's knowledge, the presence of this species always indicates disease. The major problem with selective cultures is, therefore, to answer the questions: if the patient has a positive test, what is the likelihood that disease is present ("predictive value positive"), and if the patient has a negative test, what is the likelihood that disease is absent ("predictive value negative")? The latter question is often answered by studies of the percentage of cases with a particular disease that would have been missed had only one specimen been tested. Thus, we know that discordant results can occur in approximately 10% of cultures of two separately collected specimens of throat secretions for group A streptococci, sputum specimens for *M. tuberculosis*, genital secretions for gonococci, blood for bacteria or fungi, and feces for enteric pathogens. The predictive values of results become far more complex when nonselective techniques are used for bacterial or fungal cultures of wounds, exudates, and other sites that are either normally colonized or become secondarily colonized by bacteria or fungi. In such instances, potential pathogens may often be found within the indigenous flora, and, in some instances, organisms usually considered to represent indigenous flora may, in fact, be pathogens. Whereas reports of selective cultures state whether or not the specific pathogen sought was isolated, reports of nonselective cultures may list all organisms isolated on the premise that the microbiologist is unable to accord greater clinical importance to one isolated species than to another and that the clinician should be able to interpret properly the results reported. That this is certainly not always the case was demonstrated in a nationally administered self-assessment antibiotic therapy test reported by Neu and Howry.[3]

General Guidelines for Specimen Collection

Specimens should be as representative as possible and should therefore be collected before administration of antibiotics. In certain diseases, such as those due to viruses, the etiologic agent can only be cultured during the acute phase of illness so that the diagnosis later in the course of the disease must be established by other means, such as serology.

Every effort should be made to avoid contamination of the specimen by indigenous flora through appropriate use of antiseptics for surface disinfection or the use of invasive techniques (e.g., blood culture, transtracheal or suprapubic aspiration, culdocentesis, biopsy). Specimens (e.g., sputum, urine) that traverse normally contaminated sites warrant special attention,

including instruction of patients and nursing personnel in proper collection techniques, use of screening tests in the laboratory to assess the extent of contamination, and, when appropriate, performance of quantitative or selective cultures to minimize or inhibit the growth of contaminants. All sputum specimens should be screened microscopically and another specimen requested when large numbers of squamous epithelial cells indicate excessive oropharyngeal contamination. Since the oropharynx, colon, urethra, and vagina normally harbor large numbers of diverse species of anaerobic bacteria, specimens such as sputum, oral lesions, perianal drainage, midstream urine, cervical drainage, and vaginal lesions or secretions are not suitable for anaerobic culture.

Enough of the specimen should be obtained for proper examination. Though highly convenient as a specimen collection device, the use of swabs should be limited to specimens from the skin and mucous membranes. Pus should be collected with a needle and syringe and the needle plugged for transport of the specimen to the laboratory or the contents of the syringe injected into an anaerobic transport vial. An operative specimen is obtained at some risk and considerable expense to the patient, and it is seldom possible to obtain additional material for examination if additional tests are indicated. Thus, multiple specimens should be obtained from large lesions or several small lesions. Chronic lesions typically contain few organisms, so as much specimen as possible should be obtained for examination. The yield from blood cultures is also directly related to the volume of blood cultured.

Caution should be exercised when topical anesthetics must be used to aspirate material from closed spaces since the "caines" possess antimicrobial activity. Vials of solutions (e.g., saline, lactated Ringers) used for wound irrigation often contain preservatives with bacteriostatic activity.

The number of specimens must be sufficient to minimize false-negative results. Thus, three consecutive early morning expectorated sputum specimens should be collected from patients with suspected pulmonary mycobacterioses or deep mycoses. Two to three separate blood cultures per septic episode are also necessary to detect at least 90% of patients with positive blood cultures. The presence of intestinal parasites cannot be reliably excluded without examining three purged stool specimens, and many states prohibit a food handler with salmonellosis or shigellosis from returning to work until he or she has had three consecutive culture-negative stools.

Specimens should be properly identified and accompanied by a properly

identified test request form, specifying what tests are requested and the tentative diagnosis. Communication between clinician and microbiologist is essential with problem cases and particularly when special examinations are indicated. Routine laboratory procedures may not include techniques for isolation of rarely encountered organisms.

Specimens should be transported to the laboratory as rapidly as possible to prevent loss of fastidious microorganisms and bacterial overgrowth. As a general rule of thumb, no special precautions need to be taken when the interval between collection of a specimen and processing in the laboratory is two hours or less. Transport media or devices are available for swabs, for pus or liquid specimens for anaerobic culture, and for urine and stool specimens. In some instances, refrigeration of the specimen (e.g., urine) is a simple and acceptable method of storage.

While the clinician has major responsibility for initiating requests for cultures, often the nursing service collects the specimen. It therefore behooves the microbiologist to participate regularly in in-service nursing education programs to define specimen requirements and to discuss problems regarding specimen collection and transport. More specific details about specimen collection are given elsewhere.[4]

Laboratory Procedures

In addition to guidelines for specimen collection and transport, the microbiologist needs to develop guidelines for specimen processing, including rejection criteria for unsuitable or mislabeled specimens and for inappropriate or unreasonable requests. Procedures must be selected by specimen category to permit detection of the most likely pathogens. Decisions must be made as to the extent of microbial identification, including which groups of microorganisms are appropriately reported by specimen category and whether their identification to species level is indicated. As a general rule, reports should be made of microorganisms that are clinically relevant and do not clearly constitute indigenous flora. Speciation should be carried out when it is likely to alter or to direct antibiotic therapy or when it is likely to be of epidemiological importance. The current availability of commercially prepared kits or devices allows almost any clinical laboratory to speciate the Enterobacteriaceae and many other groups of Gram-negative bacilli. The laboratory's staff must always, however, bear in mind that simply reporting a species ascribes importance to it and may influence therapy, rightly or wrongly. Further importance is ascribed to a species if its susceptibility to various antimicrobial agents is

also reported. *Staphylococcus aureus*, for example, may be isolated from the throat of anywhere from 5 to 60% of perfectly healthy children or adults, and it is not a recognized cause of pharyngitis; therefore, to report its isolation from a throat culture from a patient with pharyngitis is both irrelevant and misleading.

Tests to Guide Antimicrobial Therapy

Antimicrobial susceptibility testing. A number of tests are available to assist in antimicrobial therapy. The one most commonly performed until quite recently was the disk diffusion or Kirby-Bauer susceptibility ("sensitivity") test which provided results describing an organism as susceptible, intermediately or indeterminately susceptible, or resistant to each antibiotic tested. Despite widespread use, the derivation of these terms is not widely understood. They are based on the more or less direct relationship between a zone diameter of inhibition surrounding a disk containing a specified amount of antibiotic and the minimum concentration of the same antibiotic required to inhibit the growth of the organism. The latter is commonly referred to as the minimum inhibitory concentration or MIC. The relationship between MIC and zone diameter of inhibition is, in fact, inverse, so that if one were to determine MICs and zone diameters of inhibition for a particular antibiotic with large numbers of organisms representing a variety of species, one could plot a regression line whereby those organisms with a high MIC have a correspondingly small zone diameter of inhibition and those with a low MIC have a correspondingly large zone diameter of inhibition. Thus, within the known variability of each test, one can approximate the MIC by knowing the zone diameter of inhibition resulting from a disk diffusion test. It must be emphasized, however, that the MIC can only be approximated in this manner since the reproducibility of the MIC is ± 1 $\log_2$ dilution and the reproducibility of the zone diameter of inhibition varies to a similar degree. Contrary to popular belief, therefore, and despite the fact that the MIC is a quantitative result, the MIC is no more accurate or reproducible than the zone diameter of inhibition. Where these two tests do differ, however, is in their interpretation. One can define an organism as being "susceptible" to a particular antibiotic because its MIC is 1/4 (or less) of the average peak serum concentration attained with the usually recommended dosage of the antibiotic. By using the regression line, one can relate a concentration which is 1/4 of the average peak concentration of the antibiotic to a corresponding zone diameter of inhibition and thereby interpret as "sus-

ceptible'' any organism with a zone diameter of inhibition that equals or exceeds the specified zone diameter. Similarly, one can define as ''resistant'' any organism with an MIC that exceeds the average peak serum concentration of the antibiotic. Again, by using the regression line, one can establish a corresponding zone diameter of inhibition and thereby interpret as ''resistant'' any organism with a zone diameter of inhibition equal to or less than the specified zone diameter. What remains between these two specified zones is commonly referred to as ''intermediate'' or ''indeterminate.'' The best course of action to follow when an organism is reported in this category is to use another antibiotic to which the organism is clearly susceptible.

It should be apparent, however, that a ''susceptible'' result spans a range of MICs. For example, the MIC equivalent of ''susceptible'' for gentamicin is $\leqslant 4$ μg/ml. How much less might be important in deciding how much gentamicin to administer, particularly in an immunocompromised host or in a patient with impaired renal function, and whether to administer gentamicin alone or in combination with another antibiotic, such as ticarcillin. Under such circumstances, knowledge of the MIC is very helpful, provided—and this is the most important qualification in using MICs in general—one is familiar with gentamicin's pharmacokinetic properties and monitors its serum levels carefully.

Antibiotics normally have short half-lives, but their average peak serum levels are subject to numerous variables, including dosage, frequency and route of administration, age, excretory function, and so on. In providing a ''susceptible'' result, certain assumptions are made, such as that the dosage is that usually recommended and that if more than one dosage or route of administration can be used, the designation of ''susceptible'' applies equally under these different conditions. Again, knowledge of the MIC and a particular antibiotic's characteristics permit, under certain circumstances, adjustments in the antibiotic's administration to be made.

In recent years increasing numbers of laboratories have begun to report MICs,[5] often under the erroneous assumptions that they are more accurate and that they are of greater value to clinicians in general than the qualitative results resulting from disk diffusion tests. It did not take long for laboratories reporting MICs to discover that interpretative guidelines for MICs needed to be provided routinely on report forms and that most clinicians ignored the MICs and continued to use the interpretative results of ''susceptible,'' ''intermediate,'' or ''resistant.'' Despite this criticism of the naiveté of my colleagues in laboratories around the country, I do

think that MICs are helpful in certain very specific clinical circumstances and can be cost-effective for the laboratory when replica plating techniques are used to inoculate both biochemical tests for identification and antibiotic dilutions for determining MICs.

A novel and reasonable approach to interpreting the MIC in a more meaningful manner is the inhibitory quotient described by Ellner and Neu[6] wherein the ratios of average peak antibiotic concentrations in serum, urine, bile, or cerebrospinal fluid and MICs are reported. This approach provides the multiple of the MIC that would be achieved for each antibiotic in those body fluids, and it is, therefore, immediately apparent to the clinician which antibiotics are likely, at their recommended dosages, to be present at the site of infection at concentrations of at least four to eight times the MIC.

Antimicrobial susceptibility testing should be reserved for organisms that appear clinically important and for those with unpredictable susceptibility. The group A Streptococcus, for example, is usually of clinical importance when isolated from a throat culture; however, it remains completely predictable in its susceptibility to penicillin, and there is absolutely no justification for the laboratory to generate this information at additional cost to the patient. In most instances, panels of antibiotics appropriate for a particular organism or group of organisms are used for testing. The source or site of the organism is also usually taken into consideration. The selection of antibiotics to be tested has, however, become increasingly complex as new agents, particularly β-lactams, emerge from pharamaceutical research in bewildering numbers. Because of the cost of these newer antibiotics, the burden of their selection is shared increasingly by pharmacies, formulary committees, and other peer review groups, much to the relief of microbiologists who indirectly suffer from the promotional efforts of those harbingers of new antibiotics, the pharmaceutical representatives. Regardless, however, of how tough formulary committees become, the microbiologist is still faced with some expansion in the batteries of antibiotics to be tested on a routine basis since cross-resistance among new penicillins and cephalosporins is incomplete. Based on studies of cross-resistance, Jones[5] has published guidelines for laboratory testing of penicillins and cephalosporins (Tables I and II). These guidelines will obviously be revised as more information becomes available from other sources about cross-resistance and emerging resistance patterns.

Because resistance of methicillin (oxacillin, nafcillin, etc.) resistant

TABLE I. RECOMMENDATIONS FOR LABORATORY TESTING OF PENICILLINS*

Organism	*Test drug*	*To represent*
Enterobacteriaceae	Ampicillin	Ampicillin analogs
	Carbenicillin	Ticarcillin
	Either of:	
	Mezlocillin / Piperacillin	the other
Enterococcus	Ampicillin	Ampicillin analogs
		Apalcillin
		Azlocillin
		Mezlocillin
Other Gram-positive cocci	Penicillin	Piperacillin
	Oxacillin	Methicillin
		Nafcillin
		Cloxacillin
		Dicloxacillin
Pseudomonas	Carbenicillin	Mezlocillin
		Ticarcillin
	Any of:	
	Piperacillin / Apalcillin / Azlocillin	the others

*Based on Jones.[5]

staphylococci to cephalosporins is not detected reliably by standard disk diffusion or dilution techniques, it is suggested that resistance to oxacillin or nafcillin be used as an indicator of cephalosporin resistance of these strains, regardless of what the susceptibility test results for any of the cephalosporins are. Provided that its dosage schedule reflects its longer half-life, cefazolin has been found to be less costly than other first generation parenteral cephalosporins and has, therefore, become the first generation formulary cephalosporin in many hospitals. In such instances, the laboratory should test cefazolin instead of cephalothin, since the former is approximately four times more active than the latter against *Escherichia coli*. Some impact on cost can be effected by the laboratory in reporting the activity of second and third generation cephalosporins only in the case of resistance to the first generation cephalosporin tested.

In the past, the susceptibility of anaerobic bacteria has been highly predictable; however, recent reports of clindamycin-resistant clostridia and *Bacteroides fragilis*, β-lactamase-producing species of *Bacteroides* species other than the *B. fragilis* group, and penicillin-resistant clostridia other than *C. perfringens*[7] suggest that this situation is changing and that

TABLE II. RECOMMENDATIONS FOR LABORATORY TESTING OF CEPHALOSPORIN AND CEPHEM ANTIBIOTICS*

Organism	*Test drug*	*To represent*
	1st generation	
Staphylococcus	Cephalothin	Cefaclor Cefadroxil Cephalexin Cephaloglycin
Enterobacteriaceae	Cephalothin or Cefazolin	Cephaloridine Cephapirin Cephradine
Enterococcus	None	—
	2nd generation	
Enterobacteriaceae	Cefuroxime	Cefamandole Cefonicid Ceforanide
	Cefoxitin	Cefotetan?
Staphylococcus	None	—
Enterococcus	None	—
	3rd generation	
Enterobacteriaceae	Cefoperazone	—
	Any of: Cefotaxime Cefmenoxime Ceftazidime Ceftizoxime Ceftriaxone Moxalactam	the others
Pseudomonas	Any of: Cefotaxime Cefmenoxime Ceftriaxone Moxalactam	the others
	Cefoperazone	Cefsulodin Ceftazidime
Enterococcus	None	

*Based on Jones[5].

laboratories need to monitor susceptibility patterns of anaerobic bacteria more closely.

Bactericidal tests. Bactericidal therapy is necessary for successful treatment of infective endocarditis and generally necessary in the treatment of meningitis, osteomyelitis, and infections among immunocompromised hosts. Despite these indications for bactericidal therapy, indications for bactericidal tests are quite limited. Moreover, unlike the disk diffusion and dilution tests discussed above, standardized methods have not yet evolved for bactericidal tests. Consequently, there is considerable variation in the literature in how bactericidal tests are performed, what results are obtained, and how the results are interpreted.

There are two general categories of bactericidal tests. One is represented by the minimum bactericidal or lethal concentration (MBC or MLC) wherein a determination is made of the lowest concentration of antibiotic that is required to kill $\geq$ 99.9% of the original inoculum of a particular microorganism. This determination can be made by setting up a broth dilution test, as for determination of the MIC, and then performing quantitative subcultures of those tubes containing broth without visible growth and calculating the lowest concentration of antibiotic which killed $\geq$ 99.9% of the initiai inoculum. Alternatively, bactericidal activity can be determined by a time-kill curve wherein a specified inoculum of a microorganism is incubated with one or more fixed concentrations of an antibiotic and is then subcultured at several intervals (e.g., 4, 12, and 24 hours) to determine the rate and extent of killing. A variation of both of these methods is to test two or more antibiotics in combination to determine whether killing by the combination is significantly increased relative to that by each antibiotic alone. Synergistic combinations of antibiotics are necessary for enterococcal endocarditis and appear to be important in serious Gram-negative infections in immunocompromised hosts. Approximately 30 to 40% of enterococci from patients with endocarditis are not synergistically affected by the combination of penicillin and streptomycin but are by the combination of penicillin and gentamicin. The presence or absence of synergy in this instance, incidentally, can be readily predicted by determining whether the enterococcus is inhibited by 2,000 μg of streptomycin per ml. Hence, complete testing of numerous combinations of penicillin and streptomycin is unnecessary to assess whether or not the combination is synergistic.

The second category of bactericidal test is the serum bactericidal or lethal titer in which the serum from a patient receiving antimicrobial therapy serves as the source of antibiotic(s) for a dilution test which is otherwise performed in the same manner as the MBC. The results in this instance are reported as the highest dilution or titer of serum bactericidal or lethal to the patient's infecting microorganism. Despite the fact that this test would appear to be a marvelous way to examine *in vitro* the combined effects of antibiotics and other bactericidal factors in the patient's serum against an infecting microorganism, scant data support the prognostic value of the serum bactericidal titer in the therapy of patients with bacterial endocarditis.[8] The antibacterial activity of serum has, however, been of prognostic value in several studies by Klastersky and coworkers[9] of cancer patients with Gram-negative sepsis. In their studies, synergistic

antibiotic combinations were reflected in peak serum bactericidal titers of $\geq$1:8 and, in turn, by a more frequently favorable clinical response than when nonsynergistic combinations were used.

In general, the bactericidal activity of aminoglycosides, β-lactams, and vancomycin approximate their inhibitory activity. Hence, MBC/MIC ratios are usually $\leq$2. In recent years, however, considerable attention has been devoted to a form of antibiotic resistance described as tolerance. Tolerance is usually defined by an MBC/MIC ratio of $\geq$32. Enterococci are classic examples of microorganisms tolerant to penicillins; more recently, tolerance to penicillins and other antibiotics has been reported for viridans streptococci, including pneumococci, group B streptococci, and staphylococci. The clinical importance of tolerance has, however, remained very uncertain, perhaps because its demonstration *in vitro* is so methodologically dependent and, therefore, so variable.[10] In our experience with patients with *Staphylococcus aureus* bacteremia, tolerance of their isolates to semisynthetic penicillins has not been reflected in their serum bactericidal titers or in outcome of therapy.

Therapeutic drug monitoring. Monitoring of antibiotic concentrations in serum and other body fluids is generally recommended for antibiotics with low toxic to therapeutic ratios. In instances in which the potentially toxic level of antibiotic only slightly exceeds its therapeutic level, monitoring allows appropriate adjustments of dosages to be made, particularly in patients with diminished or changing renal or hepatic function. The major antibiotics requiring regular monitoring are aminoglycosides and vancomycin in all age groups and chloramphenicol in infants. Immunoassays and chromatographic assays are accurate, sensitive, and specific analytical methods with which determinations of concentrations of these antibiotics can be obtained and reported rapidly. Assays of β-lactam antibiotics are seldom clinically indicated and ordinarily are performed by the much slower and less specific bioassay technique.

Several principles should be remembered when monitoring antibiotic levels. First, the biologic half-life ($t_{1/2}$) of an antibiotic depends upon its volume of distribution and its clearance; therefore, dosage interval is usually based on the half-life of the antibiotic. An antibiotic with a long half-life is, consequently, administered less frequently than one with a short half-life. Second, antibiotic levels should be monitored only after the antibiotic has been administered for a long enough period of time to reach a plateau or steady state. For example, aminoglycoside levels should be monitored after the initial three to five doses have been given. Third, it

may be important to monitor both peak and trough levels, the former to determine whether therapeutic levels are present and the latter to determine whether the antibiotic is accumulating due to impaired renal function. This approach has been recommended especially for monitoring aminoglycosides. Fourth, it should be obvious from the foregoing points that the timing of blood collection must be carefully planned and that the interval between administration of a dose and collection of the specimen for assay must be documented; otherwise, the results of assays are uninterpretable. Dosage schedules are seldom precisely on time, and the times recorded for administration of dosages in nursing notes are often inaccurate. Ideally, therefore, patients requiring antibiotic monitoring should have the dose administered and the blood sample collected by the same person.

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